Supplementary Information

Methods

Cell Viability Assay

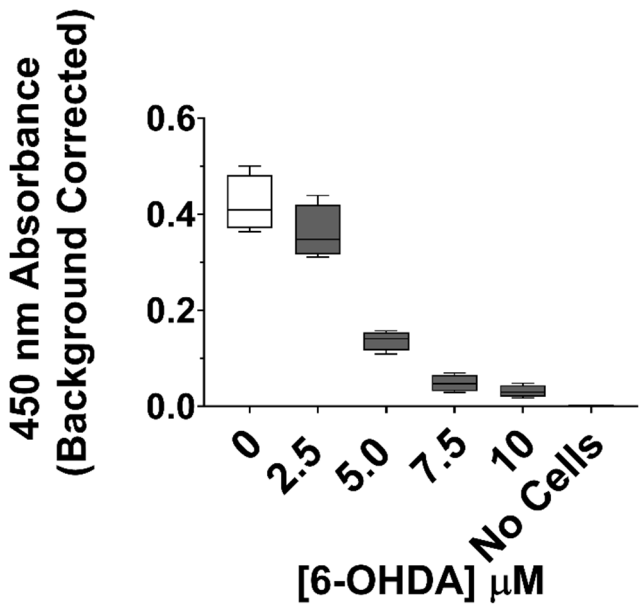
LUHMES cells were incubated for five days in differentiation media consisting of DMEM/F12 supplemented with 2 mM glutamine (VWR), 1% (v/v) N-2 supplement, 1 µg/mL tetracycline (Sigma-Aldrich, Cat# 87128-25G), 1 mM N6,2′-O-Dibutyryladenosine 3′,5′-cyclic monophosphate (db-cAMP) (Enzo Life Sciences, Farmingdale, NY, Cat# BML-CN125-0100), and 2 ng/mL glial cell line-derived neurotrophic factor (GDNF) (R&D Systems, Cat# 212-GD-010). The cells were then exposed to the indicated concentrations of 6-hydroxydopamine (Sigma Aldrich, Cat# 162957) for 24 hours. Cell viability was assessed using an XTT Cell Viability Kit using the manufacturer's instructions (Cell Signaling Technologies, Cat# 9095). A BioTek EPOCH2 platereader was used to measure absorbance at 450 nm, and absorbance values were background corrected by subtracting 450 nm absorbance measurements obtained from wells containing media and lacking cells.

Gene Expression Analysis - Quantitative Real-time PCR

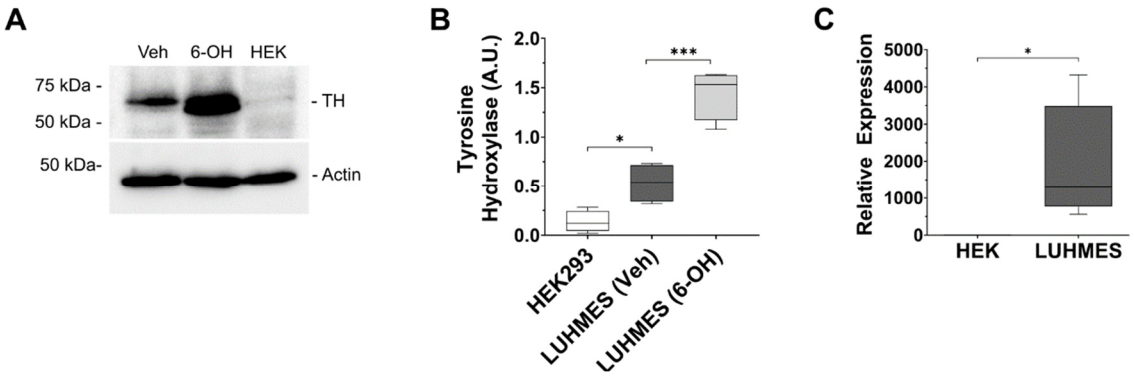
Total RNA was extracted from LUHMES cells at indicated times using Trizol (Thermo Fisher Scientific, Cat# 15596018) and a Qiagen RNeasy Mini Kit (Qiagen, Germantown, MD, ID: 74104). cDNA was prepared with Multiscribe Reverse-Transcriptase enzyme (High Capacity cDNA Archive Kit; Thermo Fisher Scientific). mRNA expression was measured by real-time quantitative PCR using predesigned TaqMan (Thermo Fisher Scientific) gene expression assays: NGFR(Hs00609976_m1), TH (Hs00165941_m1), and POLR2A (Hs00172187_m1). Samples were assayed on a Quantstudio 7 Flex Real-Time PCR System (Applied Biosystems). Relative expression was calculated using the $2^{-\Delta\Delta CT}$ method with untreated cells as controls, and expression was normalized to POLR2A RNA as an endogenous control.

Protein	% Positive	SD
$p75^{NTR}$	100	0
Tyrosine Hydroxylase	85.84	15.92
Tyrosine Hydroxylase and $p75^{NTR}$	85.84	15.92

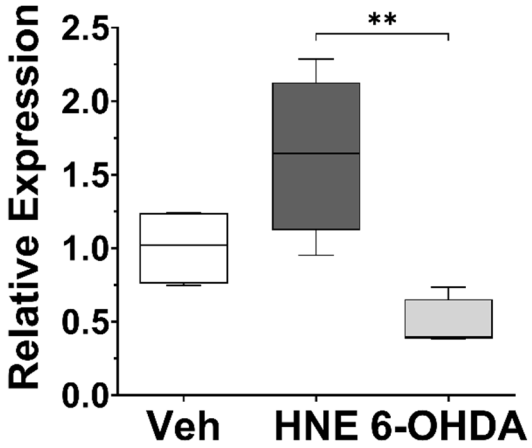
Table S1: Expression of $p75^{NTR}$ and Tyrosine Hydroxylase in Differentiated LUHMES Cells. LUHMES cells were differentiated for five days, fixed with 4% paraformaldehyde, and immunostained using antibodies specific for the $p75^{NTR}$-ECD and tyrosine hydroxylase. Image J was used to measure the mean fluorescent intensities of 316 cells from three independent experiments. The mean fluorescent intensities were compared to those generated from micrographs of control cells stained with fluorophore-conjugated antibodies in the absence of labeling with a primary antibody. Cells with a mean fluorescent intensity above the 95^{th} percentile of control cell mean fluorescent intensities were labeled as positive. Abbreviations: *$p75^{NTR}$*, p75 neurotrophin receptor; *SD*, standard deviation, *ECD*, extracellular domain.



Supplementary Figure 1: 6-hydroxydopamine Promotes a Dose-Dependent Decrease in LUHMES Cell Viability. Differentiated LUHMES cells were cultured in 96-well plates, treated with the indicated concentrations of 6-hydroxydopamine for 24 hours, and assessed for cell viability using an XTT Cell Viability Kit. A BioTek EPOCH2 platereader was used to measure absorbance at 450 nm, and absorbance values were background corrected by subtracting 450 nm absorbance measurements obtained from wells containing media and lacking cells.



Supplementary Figure 2: Expression of Tyrosine Hydroxylase in Differentiated LUHMES Cells. A; representative western blot analysis of tyrosine hydroxylase expression in lysates of LUHMES cells that were exposed to vehicle solution or 6-hydroxydopamine for 18 hours, or in untreated HEK293 cells, a non-neural cell line that was used as a negative control. Blotting for actin was used as a loading control. **B;** densitometric analysis of western blots described in A (n = 4, ANOVA with Tukey's HSD). **C;** Lysates of LUHMES cells that were differentiated for five days and lysates of HEK293 cells were assessed by quantitative PCR for expression of *TH* (n = 5 , Student's *t* test). Abbreviations: *Veh*, vehicle; *6-OH*, 6-hydroxydopamine; *HEK*, human embryonic kidney 293 cells; *A.U.*, arbitrary units; *TH*, tyrosine hydroxylase; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.



Supplementary Figure 3: Expression of *NGFR* in Differentiated LUHMES Cells Subjected to Oxidative Stress. LUHMES cells were differentiated for 5 days and treated for 8 hours with 2 µM HNE, 10 µM 6-OHDA, or vehicle solution. Lysates were then assessed by quantitative PCR for expression of *NGFR* (n = 4, ANOVA with Tukey's HSD). Abbreviations: *Veh*, vehicle; *HNE*, 4-hydroxynonenal; *6-OHDA*, 6-hydroxydopamine; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.